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(71) Applicant *(for all designated States except US)*: EMISPHERE TECHNOLOGIES, INC. [US/US]; 765 Old Saw Mill River Road, Tarrytown, NY 105491 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: GOLDBERG, Michael, M. [US/US]; 159 Lydecker Street, Englewood, NJ 07631 (US). AGARWAL, Rajesh, K. [IN/US]; 2278 Hunterbrook Road, Yorktown Heights, NY 10598 (US). CHAUDHARY, Kiran [US/US]; 8 Alicia Court, West Nyack, NY 10994 (US). MAJURU, Shingai [ZW/US]; 43 Prospect Street, Brewster, NY 10509 (US).

(74) Agents: LESSLER, Jay, P. et al.; Darby & Darby P.C., 805 Third Avenue, New York, NY 10022-7513 (US).

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(54) Title: LYOPHILIZED SOLID DOSAGE FORMS AND METHODS OF MAKING

(57) Abstract

A solid dosage form useful in the administration of active agents is provided. The solid dosage form comprises a lyophilized powder obtained from a solution comprising a delivery agent and an active agent. The solid dosage forms of the present invention have utility in the delivery of active agents to selected biological systems and in an increased bioavailability of the active agent compared to other dosage forms. Methods of preparation and administration are also provided.

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LYOPHILIZED SOLID DOSAGE FORMS AND METHODS OF MAKING

Field of the Invention

The present invention relates to a solid dosage form comprising a lyophilized powder obtained from a solution comprising a delivery agent and an active agent. The solid dosage form is suitable for oral administration of active agents to animals. Methods of preparation and administration are also disclosed.

Background of the Invention

Solid dosage forms for delivering certain active agents are well known in the art. A drug plus a diluent or excipient is milled to mix the powders to obtain a uniform mixture. The mixture is then granulated by compression or is made into a wet dough-like mixture, which is then milled to make small granules. The granules are dried in an oven or on a fluidized bed drive at temperatures of 40-80°C. The dried granules are run through a sieve to obtain particles of a uniform size. The granules are then put into capsules or compressed into tablets.

The harsh conditions of this process are of concern with protein, polypeptide or peptide active agents, such as calcitonin, insulin, human growth hormone and parathyroid hormone. The process might denature the active agent, rendering it inactive and ineffective for its intended use. Therefore, protein, polypeptide or peptide drugs are not currently made in solid dosage forms.

Recently, delivery agents have been discovered that facilitate oral and other routes of delivery of certain active agents that were previously only deliverable via injection. See, e.g., US 5,629,020; US 5,643,957; US 5,650,386; US 5,714,167; US 5,773,647; US 5,863,944; and US 5,866,536.

It has been reported that lyophilization can be used to stabilize a solution of an active agent and a delivery agent a solid which can then be reconstituted and dosed as a solution. See "Effect of Lyophilization on the Stability and In Vivo Absorption of Growth Hormone when Using PODDS™ Technology", Chaudhary et al., (abstract PDD 7403 in Pharmaceutical Research 12:9 page S-293 (September 1995) (poster presented Nov. 8, 1995 at AAPS Annual Meeting, Miami Beach, FL (November 4-9, 1995).

There is still a need to improve current delivery systems.

Summary of the Invention

A solid dosage form useful in the administration of active agents is provided. The solid dosage form comprises a lyophilized powder obtained from lyophilization of a solution comprising a delivery agent and an active agent.

The solid dosage forms of the present invention have utility in the delivery of active agents to selected biological systems and in an increased bioavailability of the active agent compared to other dosage forms.

A method of preparing the solid dosage form is provided, the method comprising:

-making a solution comprising a delivery agent and an active agent;

-lyophilizing the solution to obtain a solid powder; and

-incorporating the solid powder into a solid dosage form.

A method of administering a biologically-active agent to an animal in need of said agent comprising administering to the animal the solid dosage form is also provided.

Detailed Description of the Invention

The solid dosage forms of the present invention include an active agent and a delivery agent. These solid dosage forms may be used to deliver various active agents through various biological, chemical, and physical barriers and are particularly suited for delivering active agents which are subject to environmental degradation.

Delivery Agents

The delivery agent may be any compound that effects delivery of an active agent. The delivery may be acylated amino acids, sulfonated amino acids, ketones or aldehydes of acylated or sulfonated amino acids, or polypeptides which include any of the foregoing. Preferably, the delivery agent units comprises:

$$R\text{-}CO_2H$$

wherein:

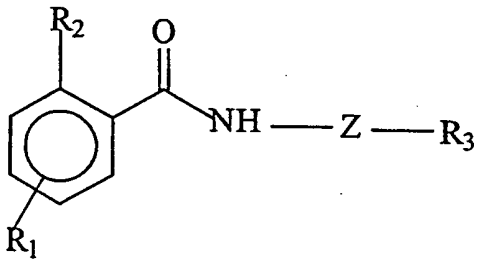
R = C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, phenyl, naphthyl, (C_1-C_{10} alkyl)phenyl, (C_2-C_{10} alkenyl)phenyl, (C_1-C_{10} alkyl)naphthyl, (C_2-C_{10} alkenyl)naphthyl, phenyl(C_1-C_{10} alkyl), phenyl(C_2-C_{10} alkenyl), naphthyl(C_1-C_{10} alkyl) and naphthyl(C_2-C_{10} alkenyl);

R may be optionally substituted with C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_1-C_4 alkoxy, -OH, -SH, -CO_2R' (where R' is H, C_1-C_4 alkyl or C_2-C_4 alkenyl), C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, heterocyclic having 3-10 ring atoms wherein the hetero atom is one or more atoms of N, O, S or any combination thereof, aryl, (C_1-C_{10} alkyl)aryl, aryl(C_1-C_{10})alkyl, or any combination thereof;

R may be optionally interrupted by O, N, S or any combination thereof; or

a salt thereof.

More preferably, the delivery agent comprises:



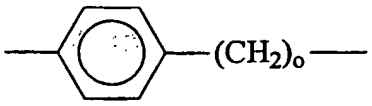
wherein:

R_1 is H, Cl, Br, Fl, CH_3, OH, OCH_3, $(CH_2)_mCH_3$ where m=1-8;

R_2 is H, OH;

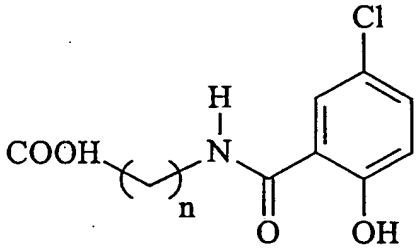
R_3 is COOH or salts thereof, NH_2, OH, Cl, Br;

Z is $(CH2)_n$ where n=1-11, and may be substituted, un-substituted, linear or branched; or

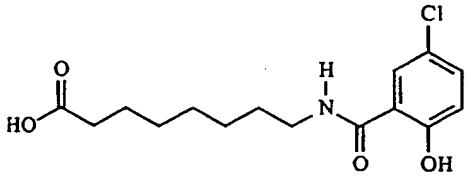


where o=1-5, and the ring and/or chain may be substituted or unsubstituted and the chain may be linear or branched.

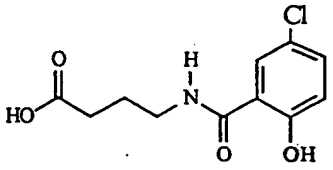
In one embodiment, the delivery agent comprises:



wherein n = 1-15, or salts thereof including but not limited to sodium salts. More preferably, the delivery agent comprises:



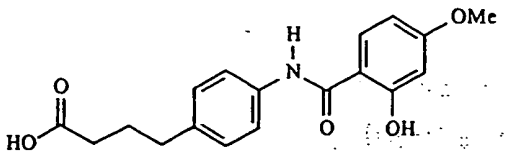
Delivery Agent 1



Delivery Agent 2

or salts thereof, including but not limited to sodium salts.

In another embodiment, the delivery agent comprises:



Delivery Agent 3

or salts thereof, including but not limited to sodium salts.

The delivery agents may be in the form of the carboxylic acid and/or their salts, including but not limited to sodium salts. In addition, poly amino acids and peptides comprising one or more of these compound may be used.

An amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring and synthetic amino acids. Poly amino acids are either peptides (which are two or more amino acids joined by a peptide bond) or are two or more amino acids linked by a bond formed by other groups which can be linked by, e.g., an ester or an anhydride linkage. Peptides can vary in length from dipeptides with two amino acids to polypeptides with several hundred amino acids. See Chambers Biological Dictionary, editor Peter M.B. Walker, Cambridge, England: Chambers Cambridge, 1989, page 215. One or more of the amino acids or peptide units may be acylated or sulfonated.

These amino acid derivatives may be derived from amino acids and can be readily prepared from amino acids by methods within the skill of those in the art based upon the present disclosure and the methods described in WO96/30036, WO97/36480, US 5,643,957 and US 5,650,386.

For example, the compounds may be prepared by reacting the single amino acid with the appropriate acylating or

amine-modifying agent, which reacts with a free amino moiety present in the amino acid to form amides. Protecting groups may be used to avoid unwanted side reactions as would be known to those skilled in the art. With regard to protecting groups, reference is made to T.W. Greene, Protecting Groups in Organic Synthesis, Wiley, New York (1981), the disclosure of which is hereby incorporated by reference.

The compound may be purified by recrystallization or by fractionation on one or more solid chromatographic supports, alone or linked in tandem. Suitable recrystallization solvent systems include, but are not limited to, acetonitrile, methanol, and tetrahydrofuran. Fractionation may be performed on a suitable chromatographic support such as alumina, using methanol/n-propanol mixtures as the mobile phase; reverse phase chromatography using trifluoroacetic acid/acetonitrile mixtures as the mobile phase; and ion exchange chromatography using water or an appropriate buffer as the mobile phase. When anion exchange chromatography is performed, preferably a 0-500 mM sodium chloride gradient is employed.

Active Agents

Active agents suitable for use in the present invention include biologically active agents and chemically active agents, including, but not limited to, pesticides, pharmacological agents, and therapeutic agents. For example, biologically or chemically active agents suitable for use in the present invention include, but are not limited to, proteins, polypeptides, peptides, and particularly small peptides; hormones, and particularly

hormones which by themselves do not pass (or which pass only a fraction of the administered dose) through the gastro-intestinal mucosa and/or are susceptible to chemical cleavage by acids and enzymes in the gastro-intestinal tract; polysaccharides, and particularly mixtures of muco-polysaccharides; carbohydrates; lipids; or any combination thereof. Further examples include, but are not limited to, the following, including synthetic, natural or recombinant sources thereof: growth hormones, including human growth hormones (hGH), recombinant human growth hormones (rhGH), bovine growth hormones, and porcine growth hormones; growth hormone-releasing hormones; interferons, including α, β and γ; interleukin-1; insulin; insulin-like growth factor, including IGF-1; heparin, including unfractionated heparin, heparinoids, dermatans, chondroitins, low molecular weight heparin, very low molecular weight heparin and ultra low molecular weight heparin; calcitonin, including salmon, eel and human; erythropoietin; atrial naturetic factor; antigens; monoclonal antibodies; somatostatin; protease inhibitors; adrenocorticotropin, gonadotropin releasing hormone; oxytocin; leutinizing-hormone-releasing-hormone; follicle stimulating hormone; glucocerebrosidase; thrombopoietin; filgrastim; prostaglandins; cyclosporin; vasopressin; cromolyn sodium (sodium or disodium chromoglycate); vancomycin; desferrioxamine (DFO); parathyroid hormone (PTH), including its fragments; antimicrobials, including anti-fungal agents; analogs, fragments, mimetics or polyethylene glycol (PEG)-modified derivatives of these compounds; or any combination thereof. In one embodiment, the active agent comprises a protein, polypeptide, or peptide; analogs, fragments, mimetics or

polyethylene glycol (PEG)-modified derivatives thereof; or any combination thereof.

Delivery systems

The solid dosage forms of the present invention comprise a delivery agent and one or more active agents and may optionally contain additives such as phosphate buffer salts, citric acid, glycols, or other dispersing agents. Stabilizing additives may be incorporated into the solution, preferably at a concentration ranging between about 0.1 and 20% (w/v).

The solid dosage unit forms may also include one or more enzyme inhibitors. Such enzyme inhibitors include, but are not limited to, compounds such as actinonin or epiactinonin and derivatives thereof. Derivatives of these compounds are disclosed in US 5,206,384. Other enzyme inhibitors include, but are not limited to, aprotinin (Trasylol) and Bowman-Birk inhibitor.

The amount of active agent is an amount effective to accomplish the purpose of the particular active agent for the target indication. The amount of active agent in the compositions typically is a pharmacologically, biologically, therapeutically, or chemically effective amount. However, the amount can be less than that amount when the composition is used in a dosage unit form that contains a plurality of delivery agent/active agent compositions or a divided pharmacologically, biologically, therapeutically, or chemically effective amount. The total effective amount can then be administered in cumulative units containing, in total, pharmacologically, biologically, therapeutically or chemically active amounts of biologically or pharmacologically active agent.

The total amount of active agent, and particularly biologically or chemically active agent, to be used can be determined by those skilled in the art. However, because the compositions may deliver active agents more efficiently than prior compositions, lower amounts of biologically or chemically active agents than those used in prior dosage unit forms or delivery systems can be administered to the subject, while still achieving the same blood levels and therapeutic effects.

Lyophilization

Lyophilization techniques are well known in the art. The delivery agent and active agent can be made up into solution and lyophilized to form a powder.

Solid Dosage Form

A solid dosage form can be a capsule, tablet or powder. The powder may be in the form of a sachet that is mixed with a liquid and administered. The solid dosage form may also be a topical delivery system, such as an ointment, cream or semi-solid. The solid dosage form contemplated may include a sustained release or controlled release system.

The lyophilized powder may be packed into capsules, or pressed into tablets, used in powder form, or incorporated into an ointment, cream or semi-solid. Methods for forming solid dosage forms are well known in the art.

The presently disclosed solid dosage forms are suitable for delivering biologically and chemically active agents, particularly in oral, intranasal, sublingual, intraduodenal, buccal, rectal, vaginal, mucosal, pulmonary, transdermal, and intramuscular systems.

The amount of lyophilized material in the present solid dosage forms is a delivery effective amount and can be determined for any particular compound or biologically or chemically active agent by methods known to those skilled in the art.

Dosage unit forms can also include any of excipients, diluents, disintegrants, lubricants, plasticizers, colorants, flavorants, taste-masking agents, sugars, sweeteners, salts, and dosing vehicles, including, but not limited to, water, 1,2-propane diol, ethanol, olive oil, or any combination thereof.

The dosage unit forms of the present invention are useful for administering biologically or chemically active agents to animals, including but not limited to poultry, such as chickens; mammals, such as cows, pigs, dogs, cats, primates, and particularly humans; and insects. The system is particularly advantageous for delivering chemically or biologically active agents which would otherwise be destroyed or rendered less effective by conditions encountered before the active agent reaches its target zone (i.e. the area in which the active agent of the delivery composition is to be released) and within the body of the animal to which they are administered. Particularly, the solid dosage forms of the present invention are useful in orally administering active agents, especially those which are not ordinarily orally deliverable.

Following administration, the active agent present in the dosage unit form is taken up into the circulation. The bioavailability of the agent is readily assessed by measuring a known pharmacological activity in blood, e.g. an increase in blood clotting time caused by heparin, or a decrease in circulating calcium levels caused by

calcitonin. Alternately, the circulating levels of the active agent itself can be measured directly.

While not wishing to be bound by theory, it is believed that the molecular level interactions of the delivery agent with the active agent is critical to effective delivery of the active agent. For example, where the active agent is a protein, water forms a protective hydration layer around the protein when it is in solution. This layer interferes with the interactions of the active agent with the delivery agent. Lyophilization of the delivery agent/active agent solution may impact the hydration layer around the protein and allow for maximum interaction between the protein and the delivery agent, thus improving delivery. It is believed that lyophilization improves interactions for non-protein active agents as well by maximizing interactions with the delivery agent.

Examples

The following examples illustrate the invention without limitation.

Example 1: Preparation of Delivery Agent 1

5-chlorosalicylamide (280 g, 1.6 mol) and acetonitrile (670 ml) were placed in a 5 liter, 4-neck, round bottomed, flask under a nitrogen atmosphere. To this stirred mixture, pyridine (161.3 g, 2.0 mol) was added over a period of 25 min. The reaction vessel was placed in an ice/water bath and portionwise addition of ethyl chloroformate was started. This addition continued over a period of one hour. When the addition was completed the

ice/water bath was removed and the reaction mixture was allowed to come to room temperature. The reaction mixture was allowed to stir for an additional one hour at room temperature before the reaction vessel was reconfigured for distillation at atmospheric pressure. The distillation that followed yielded 257.2 g of distillate at a head temperature of 78 C. 500 ml of deionized water was added to the reaction mixture that remained in the flask and the resulting slurry was vacuum filtered. The filter cake was washed with 200 ml deionized water and was allowed to dry overnight *in vacuo* at room temperature. 313.6 g (97.3%) of 6-chloro carsalam was isolated after drying. An additional batch was made using this same method and yielded 44.5 g 6-chloro-2H-1,3-benzoxazine-2,4(3H)-dione.

Sodium carbonate (194.0g, 1.8 mol) was added to 5 liter, 4-neck, round bottomed, flask containing 6-chloro-2H-1,3-benzoxazine-2,4(3H)-dione (323.1g, 1.6 mol) and dimethylacetamide (970 ml). Ethyl-8-bromooctanoate (459.0 g, 1.8 mol) was added in one portion to the stirring reaction mixture. The atmospheric pressure in the reaction vessel was reduced (550 mm Hg) and heating of the reaction mixture was started. The reaction temperature was maintained at 70 C for approximately 5 hours before heating and vacuum were discontinued and the reaction mixture was allowed to cool to room temperature overnight. The reaction mixture was vacuum filtered and the filter cake was washed with ethyl alcohol (525 ml). Deionized water (525 ml) was slowly added to the stirred filtrate and a white solid precipitated. An ice/water bath was placed around the reaction vessel and the slurry was cooled to 5 C. After stirring at this temperature for approximately 15 min the solids were recovered by vacuum filtration and the

filter cake was washed first with ethanol (300 ml) and then with heptane (400 ml). After drying overnight at room temperature *in vacuo*, 598.4 g (99.5%) of ethyl 8-(6-chloro-2H-1,3-Benzoxazine-2,4(3H)-dionyl)octanoate was obtained. An additional 66.6 g of ethyl 8-(6-chloro-2H-1,3-Benzoxazine-2,4(3H)-dionyl)octanoate was made by this same method.

Ethyl 8-(6-chloro-2H-1,3-benzoxazine-2,4(3H)-dionyl)octanoate (641 g, 1.7 mol) and ethyl alcohol (3200 ml) were added to a 22 liter, five neck flask. In a separate 5 liter flask NaOH (288.5 g, 7.2 mol) was dissolved in deionized water (3850 ml). This mixture was added to the reaction mixture contained in the 22 liter flask. A temperature increase to 40 C was noted. Heating of the reaction mixture was started and when the reaction temperature had increased to 50 C it was noted that all of the solids in the reaction mixture had dissolved. A temperature of 50 C was maintained in the reaction mixture for a period of 1.5 hr. The reaction flask was then set up for vacuum distillation. 2200 mls of distillate were collected at a vapor temperature of 55 C (10 mm Hg) before the distillation was discontinued. The reaction flask was then placed in an ice/water bath and concentrated HCl (752 ml) was added over a period of 45 min. During this addition the reaction mixture was noted to have thickened somewhat and an additional 4 liters of deionized water was added to aid the stirring of the reaction mixture. The reaction mixture was then vacuum filtered and the filter cake washed with 3 liters of deionized water. After drying *in vacuo* at room temperature 456.7 g (83.5%) of N-(5-chlorosalicyloyl)-8-aminocaprylic acid was isolated.

Example 2: Lyophilization of Salmon Calcitonin (sCT)/Delivery Agent 1 Sodium (Na) Salt

2a. Preparation of Delivery Agent 1 Sodium Salt Solution:

The percent purity of Delivery Agent 1 was determined as follows. 0.9964 g of the free acid of Delivery Agent 1 was quantitatively dissolved in 40 ml reagent methanol. 2 ml of distilled water was added to this solution after the solids were dissolved. The solution was titrated in methanol with 0.33 N sodium hydroxide using a computer controlled burette (Hamilton automatic burette available from Hamilton (Reno, NV)). A glass electrode (computer controlled Orion model 525A pH meter available from VWR Scientific, S.Plainfield, NJ) was used to monitor the pH of the solution. The solution was stirred with a magnetic stirrer.

The volume of titrant to reach the second pH inflection point was 18.80 ml. The inflection point, determined by interpolation between the two data points where the second derivative of the pH plot changed from positive to negative, occurred at pH 11.3. The purity of the free acid was determined using the following equation:

$$\% \text{ purity} = \frac{100 \times \text{ml} \times \text{Normality} \times \text{MolWt}}{1000 \times \text{Equivalents} \times \text{SampleWt}}$$

wherein

ml = milliliters of titrant
Normality = Normality of Sodium hydroxide
Mol Wt = molecular weight of the Delivery Agent 1 free acid (313.78)
Equivalents = 2 (the free acid is dibasic)
SampleWt = weight of the free acid sample being titrated

Purity was found to be 97.0%

9.3458 g delivery Agent 1 powder was weighed out. The

amount of 0.33 N sodium hydroxide needed to have a sodium hydroxide to free acid molar ratio of 1.6 was calculated using the following equation:

$$ml\ NaOH = \frac{freeAcidWt \times \%purity \times 1000 \times 1.6}{313.78 \times 100 \times Normality}$$

wherein

FreeAcidWt = weight of free acid in formulated sample
%purity = % purity of Delivery Agent 1
Normality = Normality of Sodium hydroxide
ml NaOH = amount of NaOH needed

Delivery Agent 1 and 153.3 ml 0.33 N sodium hydroxide was mixed in a Pyrex bottle. The resulting slurry was warmed in a steam bath to 60-80C. The warm slurry became a clear solution in about 15 minutes with occasional stirring. The solution was cooled to room temperature. The pH of this solution was 8.1.

2b. Preparation of sCT/Delivery Agent 1 Na Salt Solution:

The aqueous solution of Delivery Agent 1 sodium salt was filtered through a sterile, 0.45 micron cellulose acetate, low protein binding membrane on a 150 ml Corning filter (available from VWR Scientific Product, S. Plainfield, NJ). The pH of the solution was about 8.3.

Dry sCT, stored at -70C, was brought to room temperature, 18.692 mg was weighed out and dissolved in 10 ml 0.1 M mono sodium phosphate buffer solution at pH 5, with gentle mixing.

The sCT solution was added to the Delivery Agent 1 sodium salt solution with gentle mixing, taking precaution to avoid foaming or vortexing.

2c.Lyophilization of sCT/Delivery Agent 1 Na Salt Solution:

Shelves of the lyophilizer (Genesis 25 LL-800 from The Virtis Company, Gardiner, NY) were prefrozen to -45C.

Approximately 260 ml sCT/Delivery Agent 1 sodium salt solution was added to a 30cm x 18 cm stainless steel tray to give a cake thickness of about 0.48 cm. Four clean, dry thermocouple probe tips were inserted into the solution such that the probe tip touched the solution level in the center. The probes were secured with clips to the side of the tray and the trays were loaded on to the precooled shelves.

The GPC2 was programmed for the cycle listed in Table 1.

Table 1: Lyophilization Process Cycle

Step	Temperature	Pressure set point (m torr)	Time (minute)
1	- 45C	none (Prefreeze)	120
2	- 30C	300	180
3	- 20C	200	200
4	- 10C	200	360
5	- 0C	200	720
6	10C	100	540
7	20C	100	360
8	25C	100	180

When the lyophilization cycle was completed, the system cycle was terminated and the system vacuum was released. The trays were carefully removed from the shelves and the lyophilized powder was transferred into amber HDPE NALGENE® bottles (from VWR Scientific).

Using the above cycle for lyophilization, a powder with about 3% moisture content was obtained. The powder was hand packed into hard gelatin capsules (size 0EL/CS, from Capsugel, Division of Warner Lamber Co., Greenwood, SC) as needed. The filled capsules and the lyophilized powder were stored in a closed container with dessicant.

Example 3: Preparation of sCT/Delivery Agent 1 Na Salt "Fresh" Solution (unlyophilized)

Acetic anhydride (56.81 ml, 61.47 g, 0.6026 mol), 5-chlorosalicylic acid (100.00 g, 0.5794 mol), and xylenes (200 ml) were added to a 500 ml, three-neck flask fitted with a magnetic stir bar, a thermometer, and a Dean-Stark trap with condenser. The flask was heated to reflux, the reaction mixture clearing to a yellow solution around 100°C. Most of the volatile organics (xylenes and acetic acid) were distilled into the Dean-Stark trap (135-146°C). Distillation was continued for another hour, during which the pot temperature slowly rose to 190°C and the distillate slowed to a trickle to drive over any more solvent. Approximately 250 ml solvent was collected. The residue was cooled below 100 °C and dioxane was added. The reaction "set up" since it had cooled, however, in the next step heating will be applied again to make it more fluid.

A 2N sodium hydroxide (222.85 ml, 0.4457 mol) and 8-aminocaprylic acid (70.96 g, 0.4457 mol) solution was added to the solution of oligo(5-chloroasalicylic acid) (0.5794 mol) in dioxane. The reaction mixture was heated to 90°C for 5.5 hours, then shut off overnight and then restarted in the morning to heat to reflux (after restarting the heating the reaction was monitored at which time the

reaction was determined to have finished, by HPLC). The reaction mixture was cooled to 40°C. The dioxane was stripped off in vacuo. The residue was taken up in 2N sodium hydroxide and acidified. The material did not solidify. And was taken up in ethyl acetate and extracted (2x 100ml) to remove excess dioxane. The ethyl acetate layer was dried over sodium sulfate and concentrated in vacuo. The easily filtered solids were collected by filtration. The remaining material was taken up in 2N NaOH. The pH was adjusted to 4.3 to selectively isolate product from starting material. Once at pH 4.3 the solids were filtered off and then recrystallized in ethanol/water 1:1 any insoluble material was hot filtered out first. All the solids which were collected were combined and recrystallized from ethanol/water to give 52.06 g of the free acid product as a white solid.

The sodium salt solution was prepared according to the method of Example 2a using 0.2 N NaOH solution. Percent purity was calculated to be 100% using 0.5038 g of delivery agent 1 and 16.06 ml 0.2 N NaOH. The sodium salt solution was prepared using 250 ml 0.2 N NaOH and 9.4585 g delivery agent 1 prepared as above. The solution was filtered through the 0.45 micron filter.

Example 4: Oral Delivery of sCT/Delivery Agent 1 Na Salt in Rats:

Male Sprague-Dawley rats weighing between 200-250g were fasted for 24 hours and were administered ketamine (44 mg/kg) and chlorpromazine (1.5 mg/kg) 15 minutes prior to dosing. The rats were administered one of the following:

(4a) orally, one capsule of 13 mg lyophilized powder (as prepared as in Example 2) with 0.5 ml water to flush the capsule down;

(4b) orally, 1.0 ml/kg of a reconstituted aqueous solution of the lyophilized powder prepared in Example 2;

(4c) orally, 1.0 ml/kg of "fresh", unlyophilized aqueous solution of Delivery Agent 1 sodium salt as prepared in Example 3 plus sCT; or

(3d) subcutaneously, 5 µg/kg of sCT.

Doses (a), (b) and (c) contained 50 mg/kg of Delivery agent 1 Na salt and 100 µg/kg of sCT. Doses for (a) are approximate because the animals were given one capsule filled with the stated amount of powder based on an average animal weight of 250 g, whereas actual animal weight varied. This is also the case in all later examples where a capsule is dosed.

The reconstituted solution for (b) was prepared by mixing 150 mg of the lyophilized powder prepared as in Example 2 in 3 ml of water, and was dosed at 1.0 ml/mg.

The "fresh" solution for (c) was prepared from unlyophilized material using 150 mg delivery agent 1 Na salt prepared in Example 3 in 3 ml water plus 150 µl sCT stock solution (2000 µl/ml prepared in 0.1M phospate buffer, pH adjusted to 4 with HCl and NaOH. The "fresh" solution had a final concentration of 50 mg/ml delivery agent 1 Na salt and of 100 µg/ml sCT, and 1.0 ml/kg was dosed.

The subcutaneous doses were prepared by dissolving 2 mg sCT in 1 ml water. 5 µL of this solution was added to 995 µL water. This solution was dosed at 0.5 ml/kg.

Blood samples were collected serially from the tail artery. Serum sCT was determined by testing with an EIA kit (Kit # EIAS-6003 from Peninsula Laboratories, Inc., San Carlos, CA), modifying the standard protocol from the kit as follows: incubated with 50 µl peptide antibody for 2 hours with shaking in the dark, washed the plate, added serum and biotinylated peptide and diluted with 4 ml buffer, and shook overnight in the dark. Results are illustrated in Table 2, below.

Table 2. Oral Delivery of sCT/Delivery Agent 1 Na Salt in Rats

Dosage form	Delivery Agent 1 Ns Salt Dose (mg/kg)	sCT Dose (µg/kg)	Mean Peak Serum sCT ± SD (pg/ml)
(4a) capsule	50*	100*	1449 ± 2307
(4b) reconstituted solution	50	100	257 ± 326
(4c) unlyophilized solution	50	100	134 ± 169
(4d) subcutaneous	--	5	965 ± 848

* approximate dose due to variations in animal weight

Example 5: Oral Delivery of sCT/Delivery Agent 1 Na Salt in Rats

According to the method of Example 4, rats were administered one of the following:

(5a) orally, one capsule of 13 mg lyophilized powder with 1 ml water to flush the capsule down;

(5b) orally, one capsule of 6.5 mg lyophilized powder with 1 ml water to flush the capsule down;

(5c) orally, one capsule of 3.25 mg lyophilized powder with 1 ml water to flush the capsule down;

(5d) subcutaneously 5 μg/kg of sCT.

Approximate amounts of delivery agent and sCT, as well as the results, are provided in Table 3, below.

Table 3. Oral Delivery of sCT/Delivery Agent 1 Na Salt in Rats

Dosage form	Delivery Agent 1 Na Salt Dose (mg/kg)	sCT Dose (μg/kg)	Mean Peak Serum sCT ± SD (pg/ml)
(5a) capsule	50*	100*	379 ± 456
(5b) capsule	25*	50*	168 ± 241
(5c) capsule	12.5*	25*	0
(5d) subcutaneous	--	5	273 ± 320

* approximate dose due to variations in animal weight

Example 6: Preparation of Delivery Agent 2

Sodium carbonate (30g, 0.2835 mol) was added to 500 ml 3-neck, round bottomed, flask containing 6-chloro-2H-1,3-benzoxazine-2,4(3H)-dione (prepared as in Example 1, paragraph 1) (50g, 0.2532 mol) and dimethylacetamide (75 ml). Methyl-4-bromobutyrate (45.83 g, 0.2532 mol) was added in one portion to the stirring reaction mixture, and heating of the reaction mixture was started. The reaction temperature was maintained at 70 C and allowed to heat overnight. Heating was discontinued, and the reaction mixture was allowed to cool to room temperature.

The reaction mixture was vacuum filtered and the filter cake was washed with ethyl alcohol. The filter cake and filtrate were monitored by HPLC to determine where the product was. Most was washed into the filtrate, however,

product was still present in the filter cake. The filter cake was worked up to recover product to increase the final yield. The filter cake was washed first with copious amounts of water, then with ethyl acetate. The washes from the filter cake were separated and the ethyl acetate layer was next washed with 2x water, 1x brine, then dried over sodium sulfate, isolated and concentrated *in vacuo* to recover more solids (solids B). Water was added to the filtrate that had been isolated earlier and solids precipitated out. Those solids were isolated (solids A). Solids A and B were combined and transferred to a round bottom flask and 2N NaOH was added to the filtrate and heating was begun with stirring. The reaction was monitored by HPLC to determine when the reaction was done. The reaction was cooled to 25 C, stirred overnight, and concentrated *in vacuo* to remove excess ethanol. An ice/water bath was placed around the reaction vessel and the slurry was acidified. The solids were recovered by vacuum filtration and the filter cake was washed with water, dried and sent for NMR analysis.

The solids were isolated and transferred to an Erlenmeyer flask to be recrystallized. The solids were recrystallized first with methanol/water. Solids formed and were washed into a Buchner funnel, more solids precipitated out in the filtrate and were also recovered. The first solids recovered after recrystalization had formed methyl ester. All the solids were combined, 2N NaOH was added and heated again to reflux to regain the free acid. Once the ester had disappeared, as determined by HPLC, acidification of the reaction to pH 4.7 caused solids to develop.

The solids were isolated by filtration and combined with all the solids and recrystallized using a 1.5: 1.0 ratio of methanol to water. White solids precipitated out overnight and were isolated and dried to give 23.48g of N-(5-chlorosalicyloyl)-4-aminobutyric acid at a 36% yield.

It was later determined that the filter cake should have first been washed with excess ethyl alcohol to avoid having the product remain in the filter cake. From that point, the filtrate and 2N NaOH could be heated with stirring, cooled to 25C and concentrated *in vacuo* to remove excess ethanol; in an ice/water bath, the slurry acidified to pH 4.7; the solids recovered by vacuum filtration and the filter cake washed with water; solids isolated and recrystallized.

Example 7: Lyophilization of sCT/Delivery Agent 2 Na salt

Following the procedure in Example 2, a lyophilized powder of sCT/Delivery Agent 2 sodium salt was prepared and packed into capsules. 10.528 g delivery agent 2 as prepared in Example 6 was dissolved in 150 ml water. 4.72 ml 10N NaOH was added. 21.0566 mg sCT was dissolved in 10 ml phosphate buffer, and this was added to the delivery agent solution. Water was added to make the volume up to 250 ml.

Example 8: Oral Delivery of sCT/Delivery Agent 2 Na Salt in Rats

According to the method of Example 4, with the exception that the standard protocol for the EIA kit was

followed, rats were administered orally one capsule of 13 mg lyophilized powder with 0.5 ml water to flush the capsule down with the approximate amounts of Delivery Agent 2 sodium salt and sCT as set forth in Table 4 below, where results are recorded.

Table 4. Oral Delivery of sCT/Delivery Agent 2 Na Salt in Rats

Dosage form	Delivery Agent 2 Na Salt Dose (mg/kg)	SCT Dose (µg/kg)	Mean Peak Serum sCT ± SD (pg/ml)
(8a) capsule	50*	400*	1112 ± 1398
(8b) capsule	50*	800*	2199 ± 4616

* approximate dose due to variations in animal weight

Example 9: Preparation of Delivery Agent 1 for Tableting

To a clean, dry, 200 gallon glass-lined reactor 178 L of dry acetonitrile was added. The agitator was set to 100-125 RPM and the reactor contents was cooled to 9°C. 74 kg of 5-chloro salicylamide (available from Polycarbon Industries, Leominster, MA) was charged to the reactor and the charging port was closed. 47 L of dry pyridine was charged to the reactor. The slurry was cooled to 9°C prior to proceeding. Cooling was applied to the reactor condenser and valve overheads were set for total reflux. Over 2 hours, 49.7 kg of ethylchloroformate was charged to the 200 gallon reactor while maintaining the batch temperature at 14°C. (Note that ethylchloroformate can contain 0.1% phosgene and is extremely reactive with water. The reacton is highly exothermic and requires the use of a process chiller to moderate reaction temperature.) The

reactor contents was agitated for 30 minutes at 10-14°C once the ethylchloroformate addition was complete. The reactor contents was heated to 85°C over 25 minutes, collecting all distillate into a receiver. The reactor contents was held at 85-94°C for approximately 6 hours, collecting all distilled material into a receiver. The reaction mixture was sampled and the conversion (>90%) monitored by HPLC. The conversion was found to be 99.9% after 6 hours. The reactor contents was cooled to 19°C over a one-hour period. 134 L of deionized water was charged to the reactor. A precipitate formed immediately. The reactor contents was cooled to 5°C and agitated for 10.5 hours. The product continued to crystallize out of solution. The reactor slurry was centrifuged. 55 L of deionized water was charged to the 200-gallon, glass-lined reactor and the centrifuge wet cake was washed. The intermediate was dried under full vacuum (28" Hg) and 58°C for 19.5 hours. The yield was 82.6 kg 6-chloro-2H-1,3-benzoxazine-2,4(3H)-dione. This intermediate was packaged and stored so that it was not exposed to water.

In the next preparation, absolutely no water can be tolerated in the steps up to the point where distilled water is added.

222 L of dry dimethylacetamide was charged to a dry 200 gallon glass-lined reactor. The reactor agitator was set to 100-125 RPM. Cooling was applied to the condenser and valve reactor overheads were set for distillation. 41.6 kg of dry anhydrous sodium carbonate was charged to the reactor and the reactor charging port was closed. Caution was used due to some off-gassing and a slight

exotherm. 77.5 kg of dry 6-chloro-2H-1,3-benzoxazine-2,4(3H)-dione was charged to the reactor. Quickly, 88 kg of dry ethyl-8-bromooctanoate was charged to the reactor. 22-24 inches of vacuum was applied and the reactor temperature was raised to 65-75°C. The reactor temperature was maintained and the contents was watched for foaming. The reactor mixture was sampled and monitored for conversion by monitoring for the disappearance of the bromo ester in the reaction mixture by GC. The reaction was complete (0.6% bromo ester was found) after 7 hours. The vacuum was broken and the reactor contents cooled to 45-50°C. The contents was centrifuged and the filtrate sent into a second 200-gallon glass-lined reactor. 119 L of ethanol (200 proof denatured with 0.5% toluene) was charged to the first 200-gallon reactor, warmed to 45°C and the filter cake washed with warm ethanol, adding to the reaction mixture in the second 200-gallon reactor. The agitator was started on the second 200-gallon reactor. The reactor contents was cooled to 29°C. 120 L distilled water was slowly charged to the second reactor, with the water falling directly into the batch. The reactor contents was cooled to 8°C. The intermediate came out of solution and was held for 9.5 hours. The resultant slurry was centrifuged. 70 L ethanol was charged to the reactor, cooled to 8°C and the centrifuge cake was washed. The wet cake was unloaded into double polyethylene bags placed inside a paper lined drum. The yield was 123.5 kg ethyl 8-(6-chloro-2H-1,3-benzoxazine-2,4(3H)-dionyl)octanoate.

400 l purified water, USP and 45.4 kg NaOH pellets was charged to a 200 gallon glass-lined reactor and the

agitator was set to 100-125 RPM. 123.5 kg of the ethyl 8-(6-chloro-2H-1,3-benzoxazine-2,4(3H)-dionyl)octanoate wet cake was charged to the reactor. The charging port was closed. Cooling water applied to the condenser and the valve reactor overheads were set for atmospheric distillation. The reactor contents was heated to 98°C and conversion monitored by HLPC. Initially (approximately 40 minutes) the reactor refluxed at 68°C, however, as the ethanol was removed (over 3 hours) by distillation the reactor temperature rose to 98°C. The starting material disappeared, as determined by HPLC, at approximately 4 hours. The reactor contents was cooled to 27°C. 150 L purified water, USP was charged to an adjacent 200 gallon glass-lined reactor and the agitator was set to 100-125 RPM. 104 L concentrated (12M) hydrochloric acid was charged to the reactor and cooled to 24°C. The saponified reaction mixture was slowly (over 5 hours) charged to the 200-gallon glass-lined reactor. The material (45 L and 45 L) was split into 2 reactors (200 gallons each) because of carbon dioxide evolution. The product precipitated out of solution. The reaction mixture was adjusted to pH 2.0-4.0 with 50% NaOH solution (2L water, 2 kg NaOH). The reactor contents was cooled to 9-15°C. The intermediate crystallized out of solution over approximately 9 hours. The reactor slurry was centrifuged to isolate the intermediate. 50 L purified water, USP was charged to a 200-gallon glass-lined reactor and this rinse was used to wash the centrifuge wet cake. The wet cake was unloaded into double polyethylene bags placed inside a plastic drum. The N-(5-chlorosalicyloyl)-8-aminocaprylic acid was dried under vacuum (27" Hg) at 68°C for 38 hours. The dry cake

was unloaded into double polyethylene bags placed inside a 55-gallon, steel unlined, open-head drums with a desiccant bag placed on top. The dried isolated yield was 81 kg of N-(5-chlorosalicyloyl)-8-aminocaprylic acid.

Example 10: Lyophilization of sCT/Delivery Agent 1 Na Salt for Tableting

The method of Example 2 was used to prepare lyophilized powder using delivery agent 1 from Example 9. 200 g of delivery agent 1 was used. The NaOH solution was made by dissolving 42 g of 100% NaOH into 2000 ml water. The slurry was stirred at room temperature, and vacuum filtered over the 0.45 micron filter. The delivery agent 1 Na salt solution pH was about 8.6. 200 mg sCT was used.

Example 11: Preparation of sCT/Delivery Agent 1 Na Salt Tablets

Tablets of the lyophilized powder prepared in Example 10 were prepared as follows.

An instrumented Carver press (Model C, available from Carver, Wabash, Indiana) was used for tablet compression. The die used was 0.245" in diameter. The top punch was flat-faced, bevel-edged and 0.245" in diameter while the bottom punch was flat-faced, scored, bevel-edged and 0.245" in diameter. The press was capable of measuring the upper and lower punch force as well as the displacement of the upper punch. A formula for direct compression was designed as shown in Table 5 below:

Table 5:

Material	mg/tablet	mg/300 tablet batch
Lyophilized powder of SCT/Delivery Agent 1 Na Salt	100.2	30,060.0
AC-DI-SOL®	2.004	601.2
Magnesium Stearate	0.511	153.3
CAB-O-SIL®	0.205	61.5
Total weight (mg)	102.92	30,876.0

The Ac-Di-Sol® (croscarmellose sodium NF, PH.Eur., JPE, available from FMC Corporation, Pharmaceutical Division, Philadelphia, PA) and Cab-O-Sil® (fumed silica, available from Cabot Corporation, Tuscola, IL) were weighed and transferred to a mixing bottle. The bottle was then closed and secured to the arm of a sustained release apparatus set at 25 rotations per minute (RPM). The apparatus was rotated for 5 minutes to mix. The lyophilized powder of Delivery Agent 1/sCT was then added to the AC-DI-SOL®/CAB-O-SIL® mixture geometrically with a two minute mixing cycle after each addition. Magnesium stearate was then added to the above mixture and then mixing was continued for five minutes.

Approximately 103 mg of the above powder was then transferred to the die containing the lower punch. The powder was pressed down into the die using the upper punch. The upper punch was inserted and the punch die assembly was mounted onto the press. The compression was then performed. The upper punch was used to push the tablet out of the die.

Example 12: Oral Delivery of sCT/Delivery Agent 1 Na Salt in Rats - Tablets

The tablets prepared in Example 11 were pulverized and hand packed into capsules at 13 mg/capsule. Untableted, lyophilized powder as prepared in Example 10 was hand packed into capsules at 13 mg/capsule. The capsules were dosed with 1 ml water to flush them down.

Following the procedure of Example 4, with the exception that the standard protocol for the EIA kit was followed instead of the modified version, rats were administered orally one capsule with 1 ml water to flush the capsule down with the approximate amounts of Delivery Agent 1 Na salt and sCT as set forth in Table 6 below, where results are recorded.

Table 6. Oral Delivery of sCT/Delivery Agent 1 Na Salt in Rats

Dosage form	Delivery Agent 1 Na Salt Dose (mg/kg)	sCT Dose (µg/kg)	Mean Peak Serum sCT ± SD (pg/ml)
(12a) tableted powder in capsule	50*	100*	198 ± 132
(12b) untableted powder in capsule	50*	100*	197 ± 125

* approximate dose due to variations in animal weight

Example 13: Preparation of Delivery Agent 1

Delivery Agent 1 was made under similar conditions as in Example 9 in a laboratory environment.

Example 14: Lyophilization of sCT/Delivery Agent 1 Na salt

Delivery Agent 1 as prepared in Example 13 was formulated into a lyophilized powder with sCT as in Example 2 with 485 ml 0.2 N NaOH and 19.0072 g of Delivery Agent 1 in a steam bath. The final volume was 505 ml. Four separate batches were prepared from: 187, 138, 74 and 160 ml delivery agent 2 Na salt solution plus 28, 48, 40 and 360 mg sCT, respectively. The estimated amounts of delivery agent 2 Na salt were: 7, 5, 2.5 and 4.5 g, respectively.

Example 15: Oral Delivery of sCT/Delivery Agent 1 Na Salt in Rats

According to the method of Example 4, with the exception that the standard protocol for the EIA kit was followed instead of the modified version, rats were administered orally one capsule of 13 mg lyophilized powder using one of the four batches prepared in Example 14, with 1 ml water to flush the capsule down. The approximate amounts of Delivery Agent 1 Na salt and sCT are set forth in Table 7 below, where results are recorded.

Table 7. Oral Delivery of sCT/Delivery Agent 1 Na Salt in Rats			
Dosage form	Delivery Agent 1 Na Salt Dose (mg/kg)	sCT Dose (μg/kg)	Mean Peak Serum sCT ± SD (pg/ml)
(15a) capsule	50*	200*	125 ± 153
(15b) capsule	50*	400*	178 ± 354
(15c) capsule	50*	800*	546 ± 586
(15d) capsule	50*	4000*	757 ± 1234

* approximate dose due to variations in animal weight

The above mentioned patents, applications, test methods, and publications are hereby incorporated by reference in their entirety.

Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed description. All such obvious variations are within the fully intended scope of the appended claims.

What is claimed is:

1. A solid dosage form comprising a lyophilized powder obtained from a solution comprising a delivery agent and an active agent.

2. A method of making a solid dosage form comprising:

-making a solution comprising a delivery agent and an active agent;

-lyophilizing the solution to obtain a solid powder; and

-incorporating the solid powder into a solid dosage form.

3. A method of administering a biologically-active agent to an animal in need of said agent comprising administering to the animal the solid dosage form of claim 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/09412

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 9/20, 9/14
US CL : 424/464, 489
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/464, 489

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
REMINGTON'S PHARMACEUTICAL SCIENCES

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,058,623 A (HOFFMANN et al) 15 November 1977 (15.11.1977), see entire document.	1-3
X	US 4,678,812 A (BOLLIN, JR. et al) 07 July 1987 (07.07.1987), col. 8, lines 58-68.	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
AMY PULLIAM
Telephone No. (703) 308-1234

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